DETECTION OF A CARBOXYPEPTIDASE IN Aspergillus oryzae

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We have found a proteolytic enzyme with the properties of carboxypeptidase C in the preparation "orizin" (Moscow enzyme factory), which is a mixture of proteinases from Aspergillus oryzae. The carboxypeptidase activity of orizin was detected on the chromogenic substrate dinitrophenyl-glycylglycylarginine. After the hydrolysis of the dinitrophenyl-glycylglycylarginine by the orizin, the reaction products – dinitrophenyl-glycylglycine and arginine – and also the unchanged substrate can be separated by electrophoresis at pH 5.6 in pyridine acetate buffer. The dinitrophenyl-glycylglycine can be determined quantitatively after the hydrolysis of the dinitrophenyl-glycylglycylarginine by the orizin at 37°C and pH 5.6 for 1 h. For this purpose we stopped the reaction by adding 1 N HCl to pH 2. The dinitrophenyl-glycylglycine was quantitatively extracted from the reaction mixture with ethyl acetate, the unchanged substrate remaining in the aqueous phase. The dinitrophenyl-glycylglycine was reextracted from the ethyl acetate with 1% sodium bicarbonate solution, and the extinction of the resulting solution was measured at 360 nm.

The enzyme was purified by gel filtration on Sephadex G-75, and by chromatography on DEAE-cellulose acetate buffer, pH 5.6 (concentration gradient from 0.2 to 0.8 M) and QAE-Sephadex (0.1 M phosphate buffer, pH 7.0, concentration gradient from 0 to 1 M), which led to a 30-fold increase in its specific activity. The enzyme is most stable at pH 4.5-7.0 and shows activity in the pH range from 3.5 to 7.3, its optimum effect being found at about pH 5.6.

The carboxypeptidase from Aspergillus oryzae splits off the C-terminal amino acids of the following synthetic substrates: dinitrophenyl-glycylglycyl-L-arginine, acetyl-phenylalanylvaline, acetyl-phenylalanylphenylalanine, acetyl-D,L-phenylalanylglycine, and benzoyl-D,L-phenylalanyl-L-proline.

Thus, we have found a carboxypeptidase with a broad spectrum of action which differs in its specificity and optimum effect from the carboxypeptidases A and B of animal origin and is also, probably, similar to carboxypeptidase C of plant origin [1, 2] and the carboxypeptidase from bakers' yeast [3].

The results obtained, showing the extremely wide distribution of enzymes of the type of carboxypeptidase C, make an analysis of their physiological role an urgent matter [see 4].

LITERATURE CITED

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